Expression of Interferon Alfa Signaling Components in Human Alcoholic Liver Disease

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Interferon alfa (IFN-α) is currently the only well-established therapy for viral hepatitis. However, its effectiveness is much reduced (<10%) in alcoholic patients. The mechanism underlying this resistance is not fully understood. In this study, we examined the expression of IFN-α signaling components and its inhibitory factors in 9 alcoholic liver disease (ALD) and 8 healthy control liver tissues. In comparison with normal control livers, expression of IFN-β, IFN-α receptor 1/2, Jak1, and Tyk2 remained unchanged in ALD livers, whereas expression of IFN-α, signal transducer and activator of transcription factor 1 (STAT1), and p48 were up-regulated and expression of STAT2 was down-regulated. Expression of antiviral MxA a karyophilic 75 kd protein induced by IFN in mouse cells carrying the influenza virus resistance allele Mx^+ and 2′-5′ oligoadenylate synthetase (OAS) proteins was not regulated, whereas expression of double-stranded RNA-activated protein kinase (PKR) was decreased by 55% in ALD livers. Three families of inhibitory factors for the JAK-STAT signaling pathway were examined in ALD livers. Members of the suppressor of cytokine signaling (SOCS) family, including SOCS 1, 2, 3, and CIS, and the protein tyrosine phosphatases, including Shp-1, Shp-2, and CD45, were not up-regulated in ALD livers, whereas the phosphorylation of and protein levels of p42/44 mitogen-activated protein kinase (p42/44MAP kinase) were increased about 3.9- and 3.2-fold in ALD livers in comparison with normal control livers, respectively. In conclusion, these findings suggest that chronic alcohol consumption down-regulates STAT2 and PKR, but up-regulates p42/44 mitogen-activated protein kinase (p42/44MAP kinase), which may cause down-regulation of IFN-α signaling in the liver of ALD patients. (HEPATOLOGY 2002;35:425-432.)

Interferon alfa (IFN-α) therapy is the primary choice of treatment for viral hepatitis, a disease that affects millions of people worldwide.[1,2] IFN-α exerts an antiviral effect by modulating the immune system, including regulating antigen processing and presentation, and stimulating the development of specific T-helper-cell subsets.[3,4] In addition to modulation of the immune system, IFN directly eradicates the virus by stimulating production of endogenous antiviral proteins such as double-stranded RNA-activated protein kinase R (PKR), 2′-5′ oligoadenylate synthetase (OAS), and MxA, a karyophilic 75 kd protein induced by IFN in mouse cells carrying the influenza virus resistance allele Mx^+ through the Janus kinase (Jak)-signal transducer and activator of transcription factor (STAT) signaling cascade.[3,4] IFN-α/β signaling begins with ligation and dimerization of the corresponding IFN-α/β receptors (IFNαR1 and IFNαR2). Receptor dimerization induces autophosphorylation of the receptor-associated JAKs, Jak1 and Tyk2. These activated kinases also phosphorylate the receptor to recruit SH2 domain-containing STATs (STAT1 and STAT2) from the cytosol to the membrane to be phosphorylated. Once activated, STATs are released back into the cytosol for dimerization. Stat1:Stat2 heterodimers translocate into the nucleus, where they bind a third component, p48, forming the ISGF3 transcription factor complex that binds the interferon-stimulated response element to initiate transcription of IFN-α/β–specific genes.[3,4]

Several mechanisms have been implicated in down-regulation of IFN-α/β signaling. Protein tyrosine phosphatases (PTPs) such as Shp-1, Shp-2, and CD45 phosphatase have been shown to dephosphorylate IFNαR and JAKs, respectively.[5-8] A family of suppressors of cytokine signaling (SOCS) inhibitory proteins has been recently identified and shown to inhibit IFN signaling by targeting JAKs.[9] The JAK-STAT signaling pathway is also inhibited by activation of several kinases, including p42/44 MAP kinase, protein kinase C (PKC) and protein kinase A.[10-13]

Although IFN-α has been used for the treatment of viral hepatitis for more than a decade, unfortunately, more than 60% to 70% of patients respond poorly.[14-16] Most patients experience

Abbreviations: IFN-α, interferon alfa; PKR, double-stranded RNA-activated protein kinase; OAS, 2′-5′ oligoadenylate synthetase; Jak, Janus kinase; STAT, signal transducer and activator of transcription factor; MxA, a karyophilic 75 kd protein induced by IFN in mouse cells carrying the influenza virus resistance allele Mx^+; IFNαR, IFN-α receptor; PTP, protein tyrosine phosphatase; SOCS, suppressor of cytokine signaling; P42/44MAP kinase, p42/44 mitogen-activated protein kinase; PKC, protein kinase C; HCV, hepatitis C virus; ALD, alcoholic liver disease; LTPDS, Liver Tissue Procurement Distribution System; RT-PCR, reverse-transcriptase polymerase chain reaction; TNF-α, tumor necrosis factor α; IL, interleukin.

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rapid success initially with treatment, but within 6 months' post-completion of the treatment regimen, levels of hepatitis C virus (HCV) RNA and serum liver enzymes levels are again elevated. The rates of such relapse are influenced by a variety of host, viral, and treatment factors. Ethanol consumption and cirrhosis are 2 important factors that contribute to resistance to IFN-α therapy. It has been shown that IFN-α therapy is less effective in heavy drinkers,[17-20] and in patients with cirrhosis.[21,22] Currently, there is no effective therapy for HCV for these patients, and therefore, it is necessary to understand the molecular mechanisms responsible for resistance to IFN-α therapy for viral hepatitis in alcoholic patients and patients with cirrhosis to establish a more effective therapy for these patients. Here, we examined the expression of IFN-α signaling components in 9 human alcoholic liver disease (ALD) and 8 healthy human liver tissues. Expression of all IFN-α signaling components except PKR and STAT2 is not decreased in these ALD liver tissues. Moreover, p42/44 MAP kinase is highly activated in these ALD liver tissues, which may also be involved in suppression of IFN-α signaling in these patients.

Materials and Methods

Materials. Anti-STAT1, anti–phospho-ERK1/2 (Thr[202]/Tyr[204]), anti-ERK1/2, anti-JNK, and anti-p38 MAPK antibodies were obtained from NEB Bio-Lab (Beverly, MA). Anti-STAT2, anti-p48, and anti–phospho-PKR antibodies were purchased from Upstate Biotechnology (Lake Placid, NY). Anti-PKR and anti-CD45 were obtained from Transduction Laboratories (Lexington, KY).

Human ALD Specimens. The Liver Tissue Procurement Distribution System (LTPDS) program provided human ALD specimens. These patients had more than a 20-year history of heavy alcohol drinking, and had no record of viral hepatitis B or C infection. Liver pathology showed significant cirrhosis in these ALD specimens. The LTPDS program also provided normal healthy liver specimens, which were obtained from human donor livers not used for transplantation. The protocol for human subjects in the LTPDS program was approved.

Western Blotting. Western blot analysis was performed as described previously.[23] Briefly, tissues were homogenized in lysis buffer (30 mmol/L Tris [pH 7.5], 150 mmol/L sodium chloride, 1 mmol/L phenylmethylsulfonyl fluoride, 1 mmol/L sodium orthovanadate, 1% Nonidet P-40, 10% glycerol) for 3 minutes at 4°C, vortexed, and centrifuged at 16,000 rpm at 4°C for 10 minutes. The supernatants were mixed in Laemmli running buffer, boiled for 4 minutes, and then subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis. After electrophoresis, proteins were transferred onto nitrocellulose membranes and blotted against primary antibodies for 16 hours. Membranes were washed with 0.05% (vol/vol) Tween 20 in phosphate-buffered saline (pH 7.4) and incubated with a 1:4,000 dilution of secondary antibodies for 45 minutes. Protein bands were visualized by an enhanced chemifluorescence reaction (Amersham Pharmacia Biotech, Piscataway, NJ). To ensure that equal amounts of proteins were loaded, protein concentrations were carefully measured by the Bio-Rad protein assay, with bovine serum albumin as standard.

Reverse-Transcriptase Polymerase Chain Reaction. Reverse-transcription polymerase chain reaction (RT-PCR) was performed as described previously.[23] Briefly, total cellular RNA was isolated from the liver by using TRIZOL Reagent (GIBCO, Gaithersburg, MD). To ensure that equal amounts of RNA were used in RT-PCR, RNA was electrophoresed and similar densities of 28S rRNA bands were observed in these control and ALD liver samples (Fig. 1B). Five micrograms of total RNA was reverse-transcribed by random priming and incubation with 200 units of Moloney murine leukemia virus transcriptase at 42°C for 1 hour. The resulting single-stranded cDNA (5 μL) was then subjected to 30 cycles of PCR (Gradient Thermalcycler, Perkin Elmer, Foster City, CA) under standard conditions. Samples were denatured at 94°C for 3 minutes and, after the addition of the polymerase, subjected to 30 cycles of amplification each consisting of 1 minute at 94°C, 1 minute at 55°C, and 1 minute at 72°C, with a 7-minute extension at 72°C during the last cycle. Each PCR mixture (50 μL) contained the cDNA template, 1 μmol/L of primers, 200 μmol/L of dNTPs, 1.5 mmol/L $MgCl_2$, 10 mmol/L Tris/HCl (pH 9.0 at 25°C), 50 mmol/L KCl, 0.01% gelatin, and 2.5 μmol/L Taq polymerase (GIBCO). To rule out the genomic DNA contamination, PCR was also conducted with RNA in the absence of RT as the template in the reaction. No significant bands were observed in these PCR reactions.

The sequences of the primers used in the study are: IFN-α (274 bp): forward (5′ TCC ATG AGA TGA TCC AGC AG 3′) and reverse (5′ ATT TCT GCT CTG ACA ACC TCC C 3′); IFN-β (276 bp): forward (5′ TCT AGC ACT GGC TGG AAT GAG 3′) and reverse (5′ GTT TCG GAG GTA ACC TGT AAG 3′); IFNAR1 (309 bp): forward (5′ CTT TCA AGT TCA GTG GCT CCA CGC 3′) and reverse (5′ TCA CAG GCG TGT TTC CAG ACT G 3′); IFNAR2 (109 bp): forward (5′ GAA GGT GGT TAA

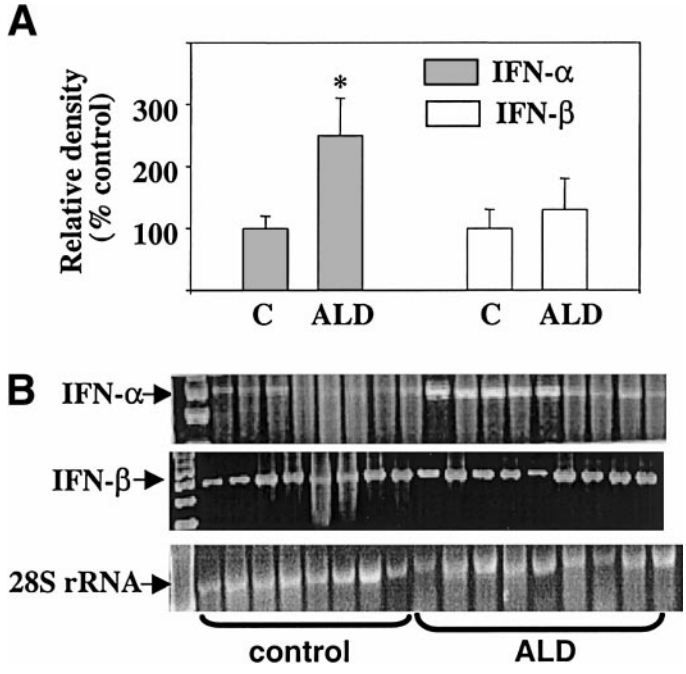

Fig. 1. Expression of IFN-α/β mRNA in ALD livers. Total RNA was isolated from 8 normal control livers and 9 ALD livers, then subjected to RT-PCR by using IFN-α or IFN-β primers, or electrophoresed (28S rRNA is shown as a loading control). (B) Ethidium bromide–stained PCR bands and 28S rRNA bands. (A) Densitometric analysis of these IFN-α and IFN-β mRNA transcripts. *$P < .01$ in comparison with control group.

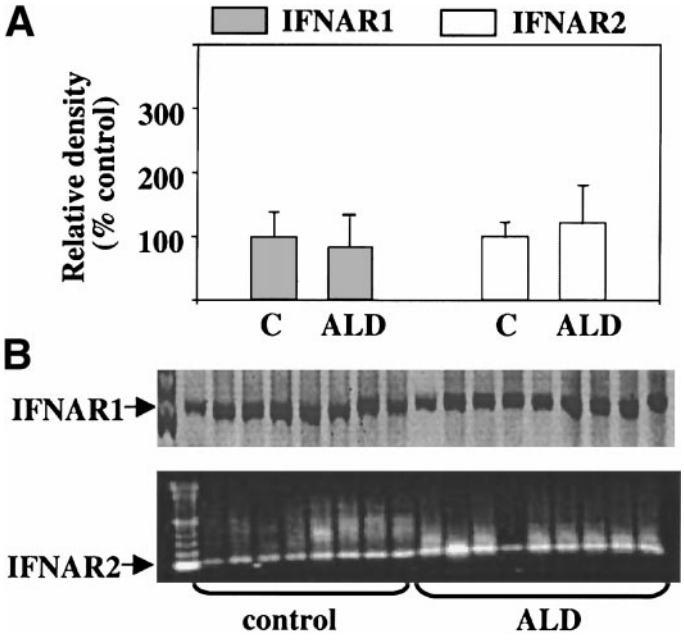

Fig. 2. Expression of IFNAR1 and IFNAR2 mRNA in ALD livers. Total RNA was isolated from 8 normal control livers and 9 ALD livers, then subjected to RT-PCR by using IFNαR1 or IFNαR2 primers. (B) Ethidium bromide–stained PCR bands. (A) Densitometric analysis of these IFNαR1 and IFNαR2 mRNA transcripts.

GAA CTG TGC 3′) and reverse (5′ CCC GCT GAA TCC TTC TAG GAC GG 3′); Tyk 2 (197 bp): forward (5′ TGC TCA GGG TCA GAT GAC AG 3′) and reverse (5′ CCT GGC CTT GGT ACT TCT CA 3′); Jak 1 (344 bp): forward (5′ GGG GCA ACT AGC AGG TGT 3′) and reverse (5′ GCA GCG TTT TAG 5′ TGA AGC TGC TGT TTC AGG 3′CAT GAA 3′); OAS3 (121 bp): forward (5′ ACT CCC AGT TCA ACA TGG 3′) and reverse (5′ TGA AGC TGC TGT TTC AGG 3′); SOCS1 (350 bp): forward (5′ CAC GCC GAT TAC CGG CGC ATC 3′) and reverse (5′ GCT CCT GCA GCG GCC GCA CG 3′); SOCS2 (300 bp): forward (5′ AAG ACA TCA GCC GGG CCG ACT A 3′) and reverse (5′ GTC TTG TTG GTA AAG GTA GTC 3′); SOCS3 (450 bp): forward (5′ GGA CCA GCG CCA CTT CTT CAC 3′) and reverse (5′ TAC TGG TCC AGG AAC TCC CGA 3′); CIS (213 bp): forward (5′ TAG TGA CTC GGT GCT GCC TAT C 3′) and reverse (5′ GTG CCT GGC TCA GTC AGA GTT G 3′).

Statistics. For comparing values obtained in 3 or more groups, one-factor ANOVA was used, followed by Tukey's *post-hoc* test, and $P < .05$ was taken to imply statistical significance.

Results

Expression of IFN-α/β and IFN-α/β Receptors in ALD Livers. To understand the molecular mechanisms underlying the high incidence of viral hepatitis infection in alcohol drinkers and ineffective IFN therapy for viral hepatitis in ALD patients, RT-PCR was performed to examine the expression of IFN-α/β and IFN-α/β receptors on liver samples from 9 ALD and 8 healthy control individuals. As shown in Fig. 1B, the expected 274-bp IFN-α fragment was found in every individual liver sample. The density of these bands in ALD liver tissues was much stronger than that in normal liver tissues. Relative quantification of these bands by a PhosphoImager showed that IFN-α mRNA transcripts were elevated about 2.5 $\pm$ 0.6-fold in ALD livers in comparison with normal control livers (Fig. 1A). In contrast, relative quantitative analyses of IFN-β mRNA (Fig. 1), IFNαR1 mRNA (Fig. 2), and IFNαR2 mRNA (Fig. 2) showed no significant difference between control and ALD individual liver tissues. Figure 1B also showed that similar densities of 28S rRNA bands were observed in these control and ALD liver samples, suggesting that equal amounts of RNA were used in RT. Taken together, these findings suggest that IFN-α mRNA transcripts are up-regulated in ALD livers, whereas IFN-β mRNA, IFNαR1 mRNA, and IFNαR2 mRNA remain unchanged.

Expression of JAKs and STATs in ALD Livers. Expression of IFN signaling components, the JAK and STAT proteins, were examined in healthy control and ALD liver samples. As shown in Fig. 3A, the 344-bp Jak1 bands and 197-bp Tyk2 bands were found in all liver samples. Quantification analyses showed that there were no significant differences between the 2 liver types (Fig. 3A). As shown in Fig. 4B, expression of both STAT1 and p48 proteins was significantly elevated in ALD livers, whereas expression of STAT2 proteins was decreased in ALD livers. Relative densitometric analyses showed that expression of STAT1 and p48 proteins increased about 2.7-fold and 2.2-fold, respectively, in ALD livers in comparison with control liver tissues. In contrast to STAT1 and p48, expression of STAT2 protein was down-regulated about 52% in ALD livers in comparison with control healthy livers.

Expression of Antiviral Factors (PKR, MxA, and OAS) in ALD Livers. Expression of 3 antiviral factors (PKR, MxA, and OAS) was examined in ALD and healthy control livers. As shown in Fig. 5B, normal healthy livers expressed high levels of 68-kd PKR protein, whereas expression of this protein was significantly

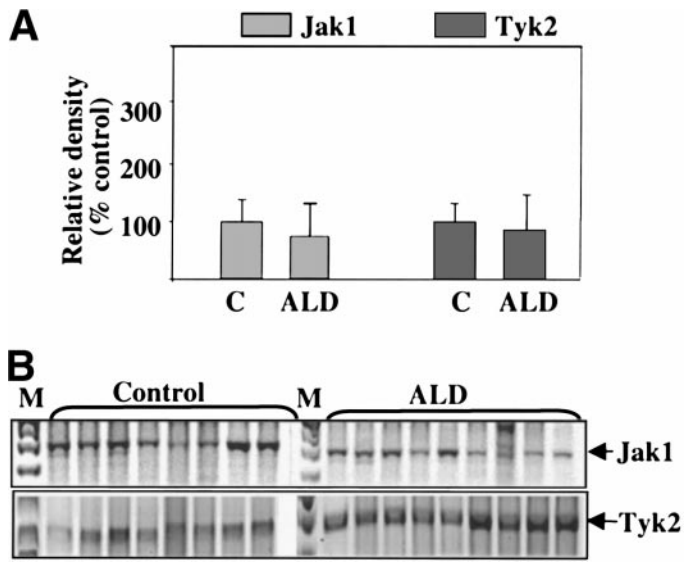

Fig. 3. Expression of Jak1 and Tyk2 mRNA in ALD livers. Total RNA was isolated from 8 normal control livers and 9 ALD livers, then subjected to RT-PCR by using Jak1 and Tyk2 primers. (B) Ethidium bromide–stained PCR bands. (A) Densitometric analysis of these Jak1 and Tyk2 mRNA transcripts.

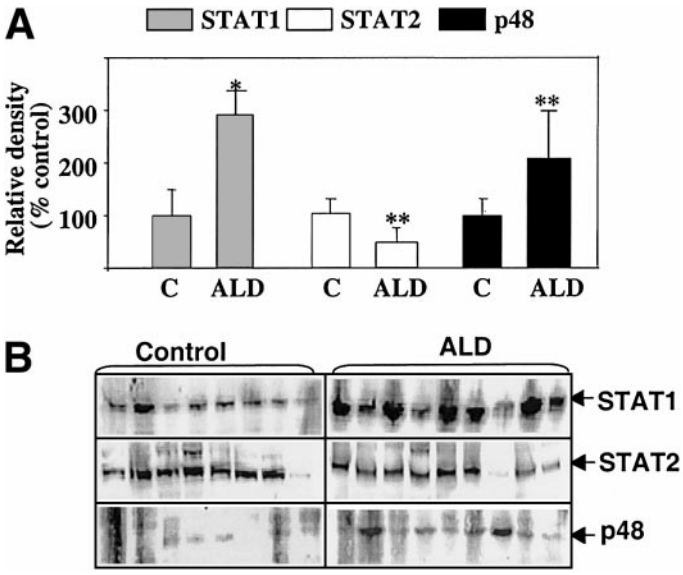

Fig. 4. Expression of STAT1, STAT2, and p48 proteins in ALD livers. Total protein extracts were isolated from 8 normal control livers and 9 ALD livers, then subjected to Western blotting by using anti-STAT1, anti-STAT2, and anti-p48 antibodies as indicated. (B) Results of Western blotting analysis. (A) Densitometric analysis of these STAT1, STAT2, and p48 protein bands. $*P < .01$; $**P < .05$ in comparison with control group.

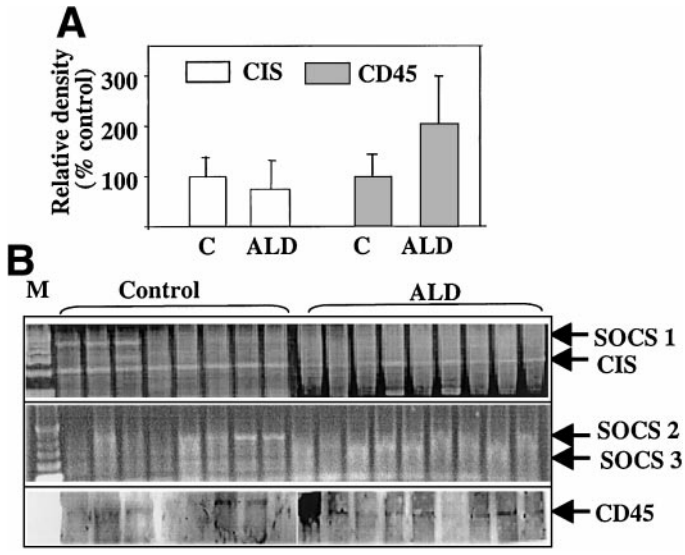

Fig. 6. Expression of SOCSs and protein tyrosine phosphatase CD45 in ALD livers. Total RNA was isolated from 8 normal control livers and 9 ALD livers, then subjected to RT-PCR by using SOCS primers as indicated, or total protein extracts were isolated and subjected to Western blotting by using anti-CD45 antibody. (B) Ethidium bromide–stained PCR bands (SOCs) and protein bands (CD45). (A) Densitometric analysis of these SOCSs and CD45 bands.

decreased in ALD livers. Densitometric analysis showed that expression of PKR protein was decreased about 55% in ALD livers in comparison with control liver tissues (Fig. 5A). Moreover, a nonspecific, faint band was observed above the 68-kd PKR signal in ALD liver samples (see explanation in Discussion). In contrast to PKR, expression of MxA mRNA remained unchanged in ALD livers (Fig. 5). The third antiviral protein, OAS, was not detected in either control healthy or ALD livers (data not shown).

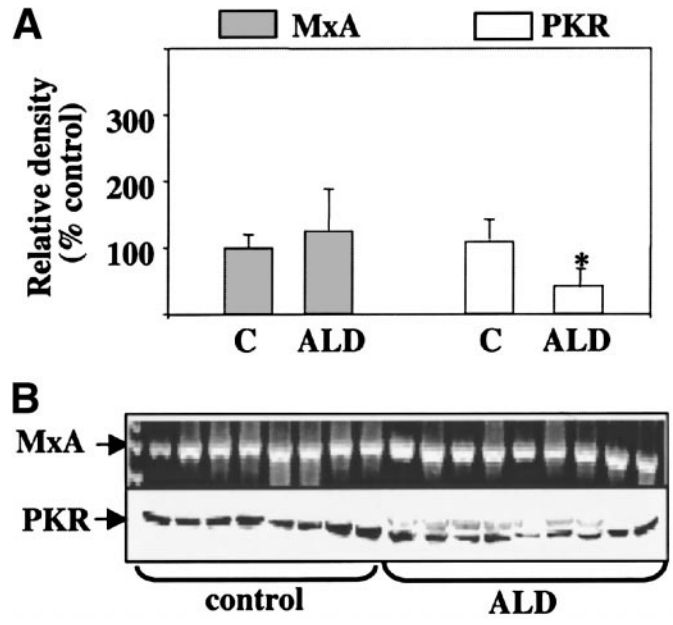

Fig. 5. Expression of antiviral MxA mRNA and PKR proteins in ALD livers. Total RNA was isolated from 8 normal control livers and 9 ALD livers, then subjected to RT-PCR by using MxA primers, or total protein extracts were isolated and subjected to Western blotting by using anti-PKR antibody. (B) Ethidium bromide–stained PCR bands (MxA) and protein bands (PKR). (A) Densitometric analysis of these MxA and PKR protein bands. $*P < .01$ in comparison with control group.

Expression of SOCS and Protein Tyrosine Phosphatases in ALD Livers. Expression of SOCS, a family of the JAK-STAT inhibitory proteins, was examined in ALD and healthy control livers. As shown in Fig. 6, of the 8 control samples, 2 individuals expressed transcripts for SOCS2. However, no SOCS2 transcripts were detected in ALD tissues. SOCS3 mRNA was undetectable across all liver samples. These data suggest that SOCS2 and SOCS3 were not actively transcribed in ALD and normal control livers. Of the 8 control livers, 3 individuals expressed transcripts for SOCS1, whereas other control and ALD livers did not express significant SOCS1. In contrast to SOCS1, 2, and 3, CIS mRNA was detected in all control and ALD livers. Densitometric analysis showed that expression of CIS was not significantly changed in ALD livers in comparison with control normal livers (Fig. 6A).

Expression of several tyrosine phosphatases was also examined in ALD and control healthy liver tissues. Western analysis showed that neither Shp-1 nor Shp-2 was detected in normal or ALD liver tissues. Low levels of CD45 were detected in control normal livers, with a slight increase of CD45 expression observed in ALD livers. Densitometric analysis showed that this increase was not significant in comparison with control normal livers (Fig. 6A).

Expression and Phosphorylation of p42/44MAP Kinase in ALD Livers. Activation of p42/44MAP kinase, which has been implicated in suppression of the JAK-STAT signaling pathway,[10-13] was examined in ALD and control healthy liver tissues. As shown in the top panel of Fig. 7B, anti–phospho-p42/44MAP kinase antibodies detected both p42 and p44 bands. Phosphorylation of p42/44MAP kinase was markedly elevated in all 9 ALD livers as compared with normal livers. The density on both bands

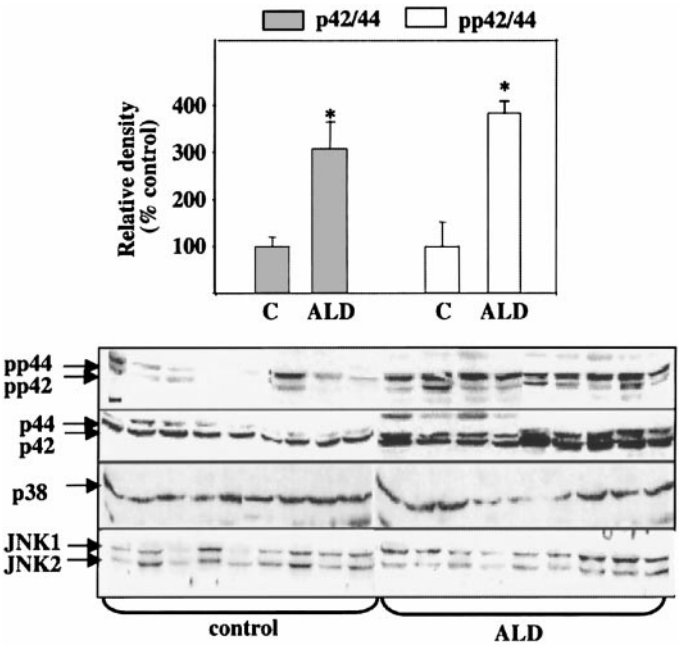

Fig. 7. Expression of p42/44MAP kinase in ALD livers. Total protein extracts were isolated from 8 normal control livers and 9 ALD livers, then subjected to Western blotting by using anti-p42/44MAP kinase, anti–phospho-p42/44MAP kinase, anti-p38MAP kinase, and anti-JNK antibodies as indicated. (B) Western blotting protein bands. (A) Densitometric analysis of these protein bands. $*P < .01$ in comparison with control group.

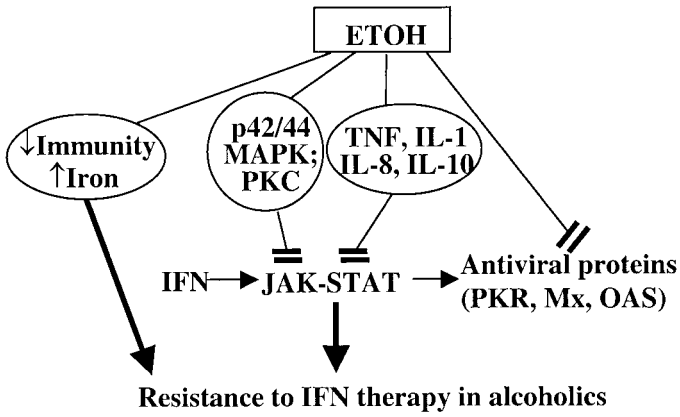

Fig. 8. Possible mechanisms are responsible for the resistance to IFN-α therapy for viral hepatitis in alcoholic patients. First, chronic ethanol consumption causes immunosuppression, such as reduction of the viral-specific–induced cytotoxic T-lymphocyte and natural-killer activity. Second, chronic ethanol consumption increases hepatic iron load, which has been involved in resistance to IFN therapy. Third, ethanol directly inhibits the IFN-α–activated signals by p42/44MAP kinase and PKC-dependent mechanisms. Fourth, chronic ethanol consumption inhibits IFN therapy by induction of a wide variety of cytokines, including TNF-α, IL-1, IL-8, and IL-10. Finally, chronic ethanol consumption down-regulates the expression of STAT2 and PKR.

was quantified, and results showed that the total phosphorylation of p44/42MAP kinase in ALD livers was about 3.9-fold over normal livers (Fig. 7). Furthermore, protein levels of p42/44MAP kinase were markedly elevated in all 9 ALD livers as compared with normal livers. The density of both bands was quantified, and results showed that the total protein levels on p44/42 MAP kinase in ALD livers were about 3.2-fold over normal livers (Fig. 7A). In contrast to p42/44MAP kinase, neither phosphorylation of nor protein levels of p38 MAP kinase and JNK were up-regulated in ALD liver tissues (the third and fourth panels of Fig. 7B).

Discussion

It has been shown that 11% to 35% of alcoholic patients are also infected with hepatitis C and B,[24] and these patients are resistant only to the well-established IFN-α therapy.[17,18,20] Several mechanisms have been implicated in resistance to IFN-α therapy caused by chronic ethanol consumption, and are summarized in Fig. 8. First, chronic ethanol consumption causes immunosuppression. The immunosuppressive effects of ethanol have been extensively studied, and it has been shown that chronic ethanol consumption causes broad immunosuppression, such as reduction of the viral-specific–induced cytotoxic T-lymphocyte and natural-killer activity.[25,26] Second, ethanol directly inhibits the IFN-α–activated signals by p42/44MAP kinase and PKC-dependent mechanisms.[27] Third, chronic ethanol consumption inhibits IFN therapy by induction of a wide variety of cytokines, including tumor necrosis factor α (TNF-α), interleukin (IL)-1, IL-8, and IL-10.[23,28-31] Fourth, chronic ethanol consumption increases hepatic iron load that has been involved in resistance to IFN therapy.[32,33] Here, we demonstrated that down-regulation of STAT2 and antiviral protein PKR, and up-regulation of p42/44MAP kinase, may also be implicated in resistance to IFN-α therapy for viral hepatitis in alcoholic patients.

To understand the molecular mechanisms underlying ineffective IFN therapy for viral hepatitis in ALD patients, we examined the expression of IFN-α signaling components and its inhibitory factors in ALD livers in comparison with normal control livers. The results are summarized in Table 1. In Table 1, we can see that most IFN-α signaling components remain unchanged in ALD livers, whereas expression of IFN-α, STAT1, and p48 was up-regulated, and expression of STAT2 was down-regulated, in ALD livers. These findings suggest that STAT2, a critical IFN-α signaling component,[3,4] is down-regulated in ALD livers, which may be involved in resistance to IFN therapy for viral hepatitis in ALD patients.

Table 1. Expression of IFN-α/β Signaling Components in ALD Livers

IFN-α Signaling	Control	ALD	Antiviral Proteins	Control	ALD
IFN-α	+	+++	PKR	+	−
IFN-β	+	+	MxA	+	+
IFNαR1	+	+	OAS	+	+
IFNαR2	+	+	**Inhibitor**		
JAK1	+	+	SOCS	+	+
JAK2	+	+	CD45	+	+
Tyk2	+	+	Shp1	+	+
STAT1	+	+++	Shp2	+	+
STAT2	+	−	pp42/44	+	++++
P48	+	++			

NOTE. Expression of mRNAs or proteins in the control liver samples was defined as "+"; expression of mRNAs or proteins in the ALD livers was defined as fold of control. Down-regulation was defined as "−."

Three major antiviral proteins have been implicated in the antiviral activity of IFN-α. The results in Table 1 show that antiviral MxA and OAS proteins remain unchanged, whereas PKR is significantly down-regulated by 55%. PKR is a serine-threonine kinase that is activated and phosphorylated by binding to dsRNA or single-stranded RNA with double-stranded regions, inactivates the initiation factor, eIF2, and consequently inhibits viral RNA translation.[4,34] Interestingly, a faint band was observed above PKR signals in ALD liver samples. Two lines of evidence strongly suggest that this faint band is a nonspecific band rather than a phosphorylated PKR form. First, we were unable to detect any phosphorylated PKR bands by using an anti–phospho-PKR antibody; second, these 70-kd faint bands in ALD liver samples were also observed in almost all of our Western blots of the samples blotted by using 40 different antibodies (data not shown). It is possible that these 70-kd faint bands represent autoantibodies in ALD liver samples, because there exist many autoantibodies against acetaldehyde-modified proteins in ALD liver samples.[35,36] It is believed that PKR acts as a major factor to initiate and amplify the antiviral defense after viral infection. After viral infection, ds-DNA virus or ssRNA virus with a double region activates PKR, followed by activation of nuclear factor-κB and consequent induction of IFNs, which induces expression of a variety of antiviral proteins, including PKR, MxA, and OAS.[4,34] Thus, low levels of PKR in ALD cannot initiate and amplify such an antiviral defense after viral infection, which may explain the higher rate of chronicity of viral infection and a consequent higher prevalence of viral markers among alcoholic patients.[19,20,24]

Two families of inhibitory factors have been implicated in down-regulation of the JAK-STAT signaling pathway. These include SOCSs and PTPs. SOCSs include SOCS1, 2, 3, and CIS. SOCS1, 2, and 3 inhibit the JAK-STAT signaling pathway by blocking Jak2 activity, whereas CIS attenuates the JAK-STAT signaling pathway by binding to phosphorylated receptors and blocking recruitment of STAT1 factors.[37,38] It has been shown that SOCS can be induced by many cytokines, including: TNF-α, IL-10, IL-6, IL-2, IL-1β, and IFN-γ.[23,29,39,40] Acute hepatic failure also up-regulates levels of SOCS1 as seen in carbon tetrachloride–treated rats.[41] Although a wide variety of cytokines that are known to induce SOCSs are elevated in alcoholic patients,[42-44] we did not detect expression of SOCSs in 9 ALD livers (Fig. 6). The reason for the lack of induction of SOCSs in ALD livers was not clear. Because SOCSs are rapidly induced proteins and rapidly degraded after synthesis,[37,38] it is possible that expression of SOCSs is transient and levels of SOCSs in ALD livers are too low to detect. Second, we examined the expression of PTPs in ALD liver tissues. Several PTPs have been implicated in down-regulation of the JAK-STAT signaling pathway. Shp-1, found mostly in hematopoietic cells, has been shown to inhibit Jak/STAT signaling induced by IL-2, IL-3, IL-4, IL-13, Epo, and IFN-α signaling.[5,7] Shp-2 has been shown to constitutively associate with JAKs and dephosphorylate the receptor and its downstream components.[6] It has been shown that Shp-2 inhibits IFN-α– and IFN-γ–induced Jak-STAT signaling[6] in mouse fibroblast cells. Recently, a transmembrane PTP, CD45, was demonstrated to inhibit cytokine and IFN-stimulated Jak-STAT signaling.[8] Although these PTPs are involved in suppression of the JAK-STAT signaling pathway, we failed to detect any significantly enhanced expression of Shp-1, Shp-2, and CD45 tyrosine phosphatases in ALD livers, suggesting that induction of these PTPs is not involved in down-regulation of IFN signaling in ALDs.

Activation of several kinases has been implicated in suppression of the JAK-STAT signaling pathway. The involvement of p42/44MAP kinase in suppression of the JAK-STAT signaling pathway has been extensively investigated.[10-13] It has been shown that p42/44MAP kinase directly phosphorylates a serine site on the STAT, followed by down-regulation of STAT binding.[13,45] Activation of p42/44MAP kinase also inhibits the JAK-STAT signaling pathway by induction of new protein synthesis.[11] Here, we demonstrated that both p42/44MAP kinase phosphorylation and expression are significantly increased in ALD livers, suggesting that increased p42/44MAP kinase could contribute, at least in part, to inhibition of IFN-α signaling and consequent suppression of IFN-α therapy for viral hepatitis in ALD patients. Ethanol was shown to increase p42/44MAP kinase activity and translocation in embryonic liver cells,[46] rat hepatocytes,[47] and in neuronal cells.[48] Thus, elevation of p42/44MAP kinase phosphorylation in ALD livers could result from direct activation by alcohol. The protein levels of p42/44MAP kinase were significantly elevated in ALD livers. Increased protein levels of p42/4MAP kinase were also reported in hepatocellular carcinoma.[49] The molecular mechanisms underlying increased expression of p42/44MAP kinase proteins in ALD and hepatocellular carcinoma livers are not known and require further studies.

In summary, we demonstrated here that STAT2, a critical IFN-α signaling component,[3,4] and PKR, an important antiviral protein downstream of IFN-α, are down-regulated in ALD livers, whereas p42/44MAP kinase is significantly elevated in ALD livers. Therefore, down-regulation of PKR and STAT2, and up-regulation of p42/44MAP kinase, may be involved in down-regulation of IFN-α signaling in ALD livers. However, whether they are also implicated in resistance to IFN therapy in HCV patients who abuse alcohol requires further studies. It would be interesting to examine whether the presence of lower levels of PKR and STAT2 proteins, and of an activation of p42/44AMP kinase, are correlated with resistance to IFN-α therapy in HCV patients who abuse alcohol as compared with those who do not have a history of alcohol drinking. If there is a correlation, stimulation of PKR and STAT2, and inhibition of p42/44MAP kinase, could be potential therapeutic targets for improving IFN therapy for viral hepatitis in these ALD patients.

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